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Immediate and delayed mortality of *Oryzaephilus surinamensis* (L.) exposed on wheat treated with diatomaceous earth: effects of temperature, relative humidity, and exposure interval☆

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Abstract

Adult *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, were exposed for 4–72 h on wheat treated with diatomaceous earth (Protect-It^{TM}), then removed and held for 1 week on untreated wheat. Beetles were exposed and held at 22, 27, and 32°C, 40, 57, and 75% r.h. (nine combinations). Mortality after the initial exposures increased as exposure interval and temperature increased, but humidity effects were inconsistent with temperature. Mortality after the one-week holding period was greater than initial mortality, and also increased as the original exposure interval and the temperature increased. At 22 and 27°C, mortality of beetles exposed from 4 to 24 h at 75% r.h. was significantly lower than mortality of beetles exposed for the same time intervals at 40 and 57% r.h. Nearly all of the beetles exposed for 72 h at all three relative humidities were dead after 1 week. The relationship between exposure interval and mortality was described by sigmoidal and linear regression for both the initial mortality and mortality 1 week after being transferred to untreated wheat. Results indicate that *O. surinamensis* is susceptible to the Protect-It^{TM} formulation of diatomaceous earth, and will continue to suffer from the effects of exposure even after they are removed from the treated environment. Published by Elsevier Science Ltd.

Keywords: Diatomaceous earth; Protect-It^{TM}; Wheat; *Oryzaephilus surinamensis*; Storage

☆ This paper reports the results of research only. Mention of a proprietary chemical or a trade name does not constitute a recommendation or endorsement by the US Department of Agriculture.

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1. Introduction

Commercial formulations of diatomaceous earth have been used in the USA to control insect pests in stored grain for more than 40 years (Korunic, 1998). Many of the older products required high application rates to give effective insect control, which affected the physical properties of the grain mass and caused mechanical problems with grain handling equipment (Korunic, 1998). Newer products can be applied at lower application rates, which alleviate some of the potential problems, but treating the entire grain mass with an insecticidal concentration of diatomaceous earth can still affect the physical properties of the grain (Jackson and Webley, 1994; Korunic et al., 1996).

In recent years, several new formulations of diatomaceous earth have been registered in the United States. One of these new formulations is Protect-It^{TM}, a marine diatomaceous earth (Quarles and Winn, 1996). Label directions specify treatment to bulk wheat as it is loaded into storage or as a surface treatment to wheat already binned. The rate for bulk grain is 0.075–0.4 mg dry dust/g (75–400 ppm, 0.15–0.80 lbs/ton), depending on the insect species and the grain moisture content. The rate for surface treatment is 0.3 mg/g to the top 45.7 cm of the grain mass (300 ppm, 0.6 lb/ton to the top 1.5 ft). Results of field and laboratory tests show applications of 75–300 ppm did not affect milling quality of grain, but the bulk density of wheat was reduced as the dosage increased (Korunic et al., 1996).

Oryzaephilus surinamensis (L.), the sawtoothed grain beetle, is a cosmopolitan insect pest of stored grains. It is an external feeder, small and highly mobile. Subramanyam et al. (1994) report results from tests in which several insect species, including *O. surinamensis*, were exposed on bulk grain that was "top dressed" with the diatomaceous earth Insecto^{TM}, and grain was sampled with traps to determine the effectiveness of Insecto^{TM}. There are few published studies in refereed journals in which *O. surinamensis* has been exposed either for short time intervals or at different temperatures and relative humidities on wheat treated with one of the new formulations of diatomaceous earth. The objectives of this test were to determine: (1) exposure intervals required to kill *O. surinamensis* exposed to wheat treated with Protect-It^{TM} at 0.3 mg/g, the label rate for surface layer treatment; and (2) effect of temperature and relative humidity on immediate and delayed beetle mortality.

2. Materials and methods

A sample of Protect-It^{TM} was obtained from Hedley Technologies (Mississauga, ON, Canada) and stored in the laboratory at ambient conditions until it was used for the test. Exposure studies were conducted at three temperatures, 22, 27, or 32°C, and three relative humidities, 40, 57, and 75% (nine temperature–humidity combinations). Humidity chambers were created by placing a waffle-type grid in the bottom of a $26 \times 36.5 \times 15$ cm plastic box, and filling the box to the level of the grid with one of three saturated salt solutions, K_2CO_3, NaBr, or NaCl, which gave relative humidities of approx. 40, 57, and 75% r.h., respectively (Greenspan, 1977). Separate tests were conducted at each temperature–relative humidity combination in random order using *O. surinamensis* obtained from pesticide-susceptible colonies maintained at 27°C, 60% r.h.

For each trial with a particular temperature–humidity combination, approx. 1.5 kg of hard red winter wheat was removed from refrigerated storage and allowed to warm at approx. 27°C. The moisture content of the wheat, as determined with a Dickey–John GAC 2000 grain analysis computer (Auburn, IL, USA), ranged from 12.6 to 12.9%. The wheat was clean and contained very little dockage or extraneous material. 35 g of wheat were put in each of thirty 40 ml cylindrical vials, and the amount of Protect-It™ necessary to treat this amount in proportion to the label rate was 10.5 mg. The wheat was treated by emptying the wheat from the vial into a 50 ml beaker, adding the dust to the wheat, hand-shaking the beaker, and pouring the wheat back into the vial. An additional six vials of untreated wheat served as untreated controls. Ten 1–2-week-old adults were put in each vial. A 1-cm hole was punched out of the vial cap and 50-mesh brass screen glued to the inside of the cap to provide air movement but still contain the insects. All 36 vials were put in a single humidity chamber containing one of the 3 salt solutions and set inside one of the three temperature incubators. These methods of treating the wheat and exposing the insects at different relative humidities were similar to those employed by Perez-Mendoza et al. (1999) for studies in which *Anisopteromalus calandrae* (Howard), a parasite of *Sitophilus oryzae* (L.), the rice weevil, were exposed on wheat treated with Protect-It™.

Beetles were exposed for 4, 8, 16, 24, 48, or 72 h, and there were five replicates plus an untreated control for each exposure interval. Upon completion of the exposure interval the vials containing the beetles were removed from the humidity chamber, beetles were classified as live or dead (initial mortality), and the treated wheat was discarded. The beetles were transferred to new vials which contained 35 g of untreated wheat with the same conditions as the treated wheat, and put back into the chamber. After an additional week the beetles were removed, classified as live or dead (1-week mortality), and the wheat and the beetles were discarded.

Temperature and relative humidity inside the plastic boxes were monitored using HOBO data recorders (Onset Computer Corporation, Pocasset, MA, USA). The test was analyzed using the General Linear Models Procedure of SAS (SAS Institute, 1987), with beetle mortality as the response variable and temperature, relative humidity, and exposure interval as main effects. Initial mortality and delayed mortality (1-week mortality) were repeated measures. Control mortality was rare, and when corrections for mortality were necessary they were done using Abbott's (1925) formula. Lack-of-fit tests (Draper and Smith, 1981) were conducted using Table curve 2D software (Jandel Scientific) to determine the amount of variation that could be explained by any model fitted to the data (maximum R^2), and the amount of variation explained by the given equation (R^2), and to fit appropriate regression curves to the data.

3. Results

The main effects, temperature ($F = 269.8$, d.f. = 2, 432), relative humidity ($F = 14.5$, d.f. = 2, 432), exposure interval ($F = 463.1$, d.f. = 5, 432), and the repeated measure, initial vs delayed mortality ($F = 67.3$, d.f. = 1, 97) were all significant at the 0.01 level. Mortality of *O. surinamensis* generally increased as exposure interval and temperature increased, and although mortality within a given temperature was occasionally significant with respect to relative humidity, the effects were not consistent. Since humidity effects were not consistent and varied with temperature, data were combined and plotted for each temperature with exposure interval as the independent variable. Data for all three temperatures were described by sigmoidal regression (Table 1 and Fig. 1), and the plotted lines show the progressive increase in mortality with increasing temperature [Fig. 1(A)–(C)].

Mortality of *O. surinamensis* after 1 week on untreated wheat was usually greater than the initial mortality, indicating the beetles continued to be affected by exposure to diatomaceous earth even after they were removed from the treated wheat and placed on untreated wheat. This was especially evident for beetles exposed for 4–24 h. Mortality after the one-week holding period increased with both the initial exposure interval and the temperature at which beetles were exposed and held. At 22°C, no humidity comparisons were significant ($P \geq 0.05$), therefore data were combined with the original exposure interval as the independent variable. Mortality of beetles originally exposed for 24 h or less did not exceed 27%, but as the exposure interval increased to 48 and 72 h, mortality increased to 66 and 76%, respectively (Fig. 2). Data were described by sigmoidal regression (Table 2 and Fig. 2). At 27 and 32°C, mortality of beetles exposed for 4–24 h at 75% r.h. was significantly lower ($P < 0.05$) than mortality of beetles exposed for the same time intervals at 40 and 57% r.h. However, approx. 80–100% of the beetles exposed for 48 and 72 h were dead after 1 week [Fig. 3(A)–(F)]. Data for each humidity at 27 and 32°C were described by sigmoidal and linear regression, and all except one of the adjusted R^2 values for all the sigmoidal equations were above 90% (Table 2 and Fig. 3).

4. Discussion

Mixed reports are found in the literature regarding the susceptibility of *O. surinamensis* to different formulations of diatomaceous earth. Golob (1997) states that secondary beetle pests of stored grains are more susceptible to diatomaceous earth than internal feeders such as *S. oryzae* or *R. dominica* (F). However, Korunic (1998) cites studies with several genera which

Table 1
Sigmoid equations[a] describing mortality of *O. surinamensis* exposed for 4–72 h on wheat treated with diatomaceous earth (Protect-It™) at the rate of 10.5 mg/35 g of wheat (300 ppm). Beetles were exposed and held at 22, 27, and 32°C; data were combined for 40, 57, and 75% relative humidity (r.h.). For all equations Y = % mortality, x = exposure interval (h)

Temp. (°C)	Equation parameters ± SE			R^2	Maximum R^2	% of maximum
	a	b	c			
22	81.4 ± 13.4	53.7 ± 5.7	11.7 ± 3.0	0.79	0.79	100.0
27	93.9 ± 4.2	34.6 ± 2.0	8.9 ± 1.1	0.88	0.89	98.9
32	93.2 ± 2.8	21.5 ± 0.8	5.3 ± 0.8	0.87	0.87	100.0

[a] Sigmoid equations, $y = a/\{1+\exp[-(x-b)/c]\}$.

indicate that *Cryptolestes* spp are very susceptible to diatomaceous earth, *Sitophilus* are less sensitive, followed by *Oryzaephilus, Rhyzopertha*, and *Tribolium*. The results of this study show that *O. surinamensis* was susceptible to the Protect-It$^{\text{TM}}$ formulation of diatomaceous earth. At least 95% of the insects were dead in 3 days after exposure at 27 and 32°C; 40, 57, and 75% r.h. on wheat treated at the rate of 300 ppm. All were dead 1 week after they were removed from the treated wheat and put on untreated wheat, indicating that *O. surinamensis* continued to be affected by the exposure to Protect-It$^{\text{TM}}$. In studies with Insecto$^{\text{TM}}$, another new formulation of diatomaceous earth, all *Cryptolestes ferrugineus* (Stephens) and *C. pusillus* (Schoenherr) were dead after 2 days of continuous exposure on corn treated at the rate of 500

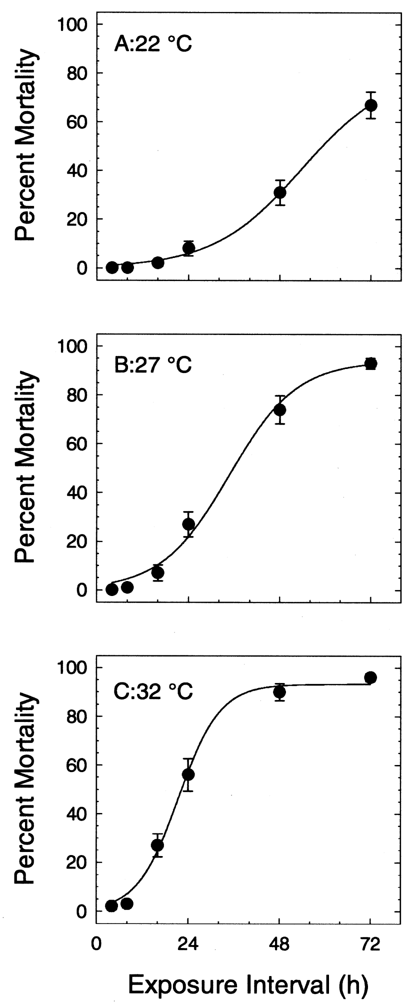

Fig. 1. Mortality (mean ± SEM) of *O. surinamensis* exposed to Protect-It$^{\text{TM}}$ for 4–72 h on wheat held at 22, 27, and 32°C; data for 40, 57, and 75% relative humidity combined for each temperature. Curve-fit lines are from the equations in Table 1. For all equations Y = % mortality, x = exposure interval (h).

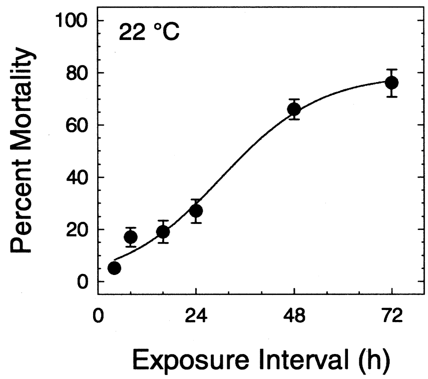

Fig. 2. Mortality of *O. surinamensis* after they were exposed at 22°C, then removed from the exposure arenas and held for 1 week at the same temperature and relative humidity (40, 57, and 75%, r.h.) at which they were exposed. Data for 40, 57, and 75% relative humidity were combined for each temperature. Curve-fit lines are from the equations in Table 2. For all equations $Y = \%$ mortality, x = exposure interval (h).

ppm, but 7 days of exposure were required to give 100% kill of *O. surinamensis* (Subramanyam et al., 1994).

Mixed results have been reported regarding the efficacy of diatomaceous earths when stored-product beetles have been exposed at different temperatures. In studies where beetles were exposed at 20 and 30°C on wheat treated with silica aerogel, *S. granarius* (L) and *R. dominica* were more susceptible at 30 than 20°C, but the reverse was true for *T. castaneum* (Herbst) (Aldryhim, 1990, 1993). Fields and Korunic (2000) also show decreased susceptibility of *T. castaneum* exposed at 30 versus 20°C on wheat treated with several different formulations of diatomaceous earth, but *C. ferrugineus* was more susceptible as temperature increased. Mixed

Table 2
Sigmoid equations[a] describing mortality of *O. surinamensis* after they were removed from the exposure arenas and held for 1 week at the same temperature (22, 27, and 32°C) and relative humidity (40, 57, and 75%, r.h.) at which they were exposed. The independent variable (x) is the exposure interval. For all equations $Y = \%$ mortality, x = exposure interval (h); data are combined for 22°C

		Equation parameters±SE					
Temp. (°C)	r.h. (%)	*a*	*b*	*c*	R^2	Maximum R^2	% of maximum
22	–	79.2 ± 5.4	30.1 ± 3.3	12.2 ± 2.1	0.74	0.75	98.6
27	40	90.0 ± 3.6	5.4 ± 1.1	5.8 ± 1.6	0.74	0.77	96.1
	57	84.0 ± 6.9	8.8 ± 2.6	7.5 ± 3.1	0.52	0.57	91.2
	75	98.7 ± 4.0	18.1 ± 1.1	5.9 ± 0.9	0.91	0.93	97.8
32	40	92.8 ± 5.4	192.7 ± 45.3[b]		0.39	0.58	67.2
	57	101.4 ± 4.8	3.8 ± 1.9	8.1 ± 2.7	0.63	0.67	94.0
	75	96.1 ± 4.7	19.4 ± 1.8	8.6 ± 1.7	0.87	0.88	98.8

[a] Sigmoid equations, $y = a/\{1 + \exp[-(x-b)/c]\}$.
[b] Linear equation $y = a-(b/x)$.

results were reported for *S. oryzae*, depending on the specific formulation. In the current test, mortality of *O. surinamensis* directly increased as temperature increased. Insect activity usually increases as temperature increases, therefore *O. surinamensis* could have been coated with more of the diatomaceous earth as they became more active.

Reviews of studies in which stored-product beetles have been exposed to inert dusts and diatomaceous earths report a general decrease in efficacy with an increase in grain moisture content or relative humidity (Golob, 1997; Korunic, 1998; Fields and Korunic, 2000). One published study with *O. surinamensis* reports a 6–8-fold increase in the LC_{50} as grain moisture content increases from 11 to 16%, but studies were done only at 25°C and a 7-day exposure

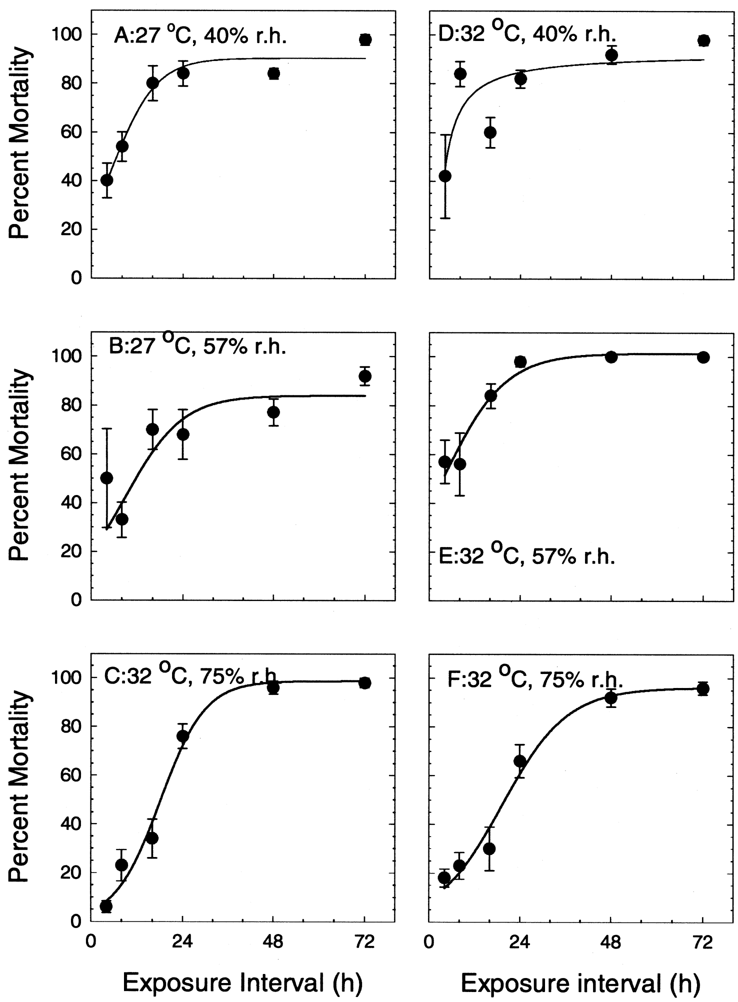

Fig. 3. Mortality of *O. surinamensis* after they were exposed at 27 and 32°C, then removed from the exposure arenas and held for 1 week at the same temperature and relative humidity (40, 57, and 75%, r.h.) at which they were exposed. Curve-fit lines are from the equations in Table 2. For all equations $Y = \%$ mortality, x = exposure interval (h).

interval (Le Patourel, 1986). Results from my tests show that at each exposure interval and temperature, mortality was often lower at 75% r.h. compared to 40 and 57% r.h., but results between 40 and 57% r.h. were variable and inconclusive. The Protect-It^{TM} formulation may have been so effective towards *O. surinamensis* that there was essentially no difference in toxicity at the two lower humidities.

Insect pests of stored grain often enter a bin after it is filled, and infestations can be concentrated in the top surface of the grain mass (Hagstrum, 1987, 1989). Using diatomaceous earth as a surface application could reduce some of the problems caused by treating bulk grain, while targeting the specific portion of the bin where infestation is likely to occur. However, when any insecticide, including diatomaceous earth, is used as a surface treatment in stored grain, insects can potentially escape the insecticide by moving through the treated layer. Although mobile insects such as *O. surinamensis* could potentially move through a surface application of diatomaceous earth within a grain bin without being killed, results of this study show that some delayed mortality will occur after the beetles leave the treated environment.

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